*[D 7050, 8010; Q 1230.]

"Studies on Enzyme Action. II.—The Rate of the Change, conditioned by Sucroclastic† Enzymes, and its Bearing on the Law of Mass Action." By EDWARD FRANKLAND ARMSTRONG, Ph.D., Salters' Company's Research Fellow, Chemical Department City and Guilds of London Institute, Central Technical College. Communicated by Professor H. E. ARMSTRONG, F.R.S. Received April 5,—Read April 28, 1904.

*D 0070, Q 0070 } Nomenclature of enzymes.
D 1820 Milk sugar and maltose, hydrolysis by enzymes.
D 8010 Emulsin, lactase, maltase—kinetics of their action.

Although it is now universally recognised that enzymes play a most important part in animal and plant metabolism and that they even condition synthetic changes, most of the work done has been of a qualitative character; in only a few cases has the nature of the action been precisely determined—and the results arrived at in these few cases, if not discrepant, cannot easily be harmonised at first sight.

C. O'Sullivan and Tompson‡ were the first to study the action of enzymes quantitatively. They came to the conclusion that the action of invertase on cane sugar takes place in accordance with the ordinary theory of mass action, a theory which involves the assumption that, throughout an interaction, the amount of change taking place in a given interval of time is always the same proportion of the material remaining unchanged—so that the course of change is expressible by a logarithmic curve.

Duclaux§ subsequently contended that the rate of change during the early period is directly proportional to the time, although afterwards, under the influence of the products of hydrolysis, it follows the logarithmic law. If this suggestion that the action follows the law

* [Index supplied by communicator, classified according to the schedule of the International Catalogue of Scientific Literature.]

† [Attention was directed by me in 1890 to the fact that "the terms amylolytic, proteolytic, etc., are confusing to the student who has learnt that electrolysis signifies splitting up by means of electricity and hydrolysis splitting up by means of water—not the splitting up of electricity or of water." ("The Terminology of Hydrolysis, especially as affected by Ferments," 'Chem. Soc. Trans.,' 1890, p. 528.) Unfortunately such terms are still generally used. To avoid the difficulty, I would suggest that enzymes should be spoken of as sucroclastic, glucosidoclastic, amyloclastic, proteoclastic, lipoclastic, etc., according as they condition the hydrolysis of disaccharides, glucosides, starch, proteids, fats, etc.—H. E. A.]

‡ 'Chem. Soc. Trans.,' 1890, vol. 57, p. 843.

§ 'Ann. Inst. Pasteur,' 1898, vol. 12, p. 96.

only when it is modified by the products of change be accepted, it follows that the interaction does not take place at any time in accordance with the law of mass action.

Somewhat later, an elaborate investigation of the action of enzymes was carried out by Victor Henri, whose work is summarised in his 'Lois générales des diastases,' Paris, 1903. Henri found that the velocity coefficient K, calculated on the assumption that the logarithmic law was applicable, steadily increased in value as the action proceeded. He attributed the increase to the influence of the products of change $\left(\frac{x}{S}\right)$ and took these into account by writing $K_1\left(1+\epsilon\frac{x}{S}\right)$ in place of K in the equation of mass action, which thus becomes

$$\frac{dx}{dt} = K_1\left(1+\epsilon\frac{x}{S}\right)(S-x).$$

This expression was derived from Ostwald's 'Lehrbuch.'* It may be pointed out that it is there put forward as applicable to cases of change in which the products may be assumed to have an *accelerating influence,* and that, as a matter of fact, it is the equation to a curve showing a change in direction corresponding to a rise to a maximum velocity and a subsequent fall in the rate. Henri deduced from his results the value $+1$ for ϵ, so that the equation became

$$2K_1 = \frac{1}{t}\log\frac{S+x}{S-x}.$$

In 1902, Adrian Brown,† besides arriving at the conclusion that hydrolysis is effected at a more rapid rate than is indicated by the law of mass action, also showed that on varying the concentration an approximately *constant weight*—not as the law of mass action requires a *constant proportion*—of sugar is hydrolysed in a given time. It is necessary to bear in mind, however, that in his experiments, the proportion of the total sugar hydrolysed in no case exceeded one-fifth, so that in reality the comparison was made only during the earlier stage of the hydrolysis. To explain the somewhat remarkable result to which he was led, Adrian Brown assumed that not only is a compound of enzyme and sugar formed but that this persists during a really appreciable interval of time: consequently a molecule of the enzyme can effect only a limited number of complete molecular changes in unit time; whatever the available mass may be, no increase in the amount of substance changed is possible. On the other hand, in dilute solutions in which the proportion of sugar to enzyme falls below a certain maximum, the amount of sugar hydrolysed should be directly proportional to the amount present—and this Adrian Brown proved to be the case.

* 2nd edition, vol. 2, p. 264.

† 'Chem. Soc. Trans.,' vol. 81, p. 373.

Similar results were obtained by Horace Brown and Glendinning* in the case of the hydrolysis of starch under the influence of diastase. Stress was laid by these observers on the fact that, at first, equal amounts of starch are hydrolysed in equal intervals of time and that subsequently the change follows the usual logarithmic law.

It is noteworthy, however, that when this logarithmic law is applied either to Henri's results or to those of Adrian Brown for invertase or to those of Horace Brown and Glendinning for diastase, reckoning from the commencement of the change, a series of increasing values is obtained for K, whereas when Henri's equation is used K_1 is a constant in each case. It would thus appear that the action of the two enzymes follows the same fundamental law.

The nature of the change effected by enzymes has been fully discussed by Horace Brown and Glendinning. Starting from the conception that hydrolysis is preceded by a combination of the hydrolyte with the enzyme, on the assumption that the concentration of the added enzyme is very small in relation to the initial concentration of the sugar, they point out that, in the earlier stages of the hydrolysis, the amount of sugar in unit volume will be very large compared with the amount of the combination of sugar with enzyme: consequently, so long as the concentration of the unaltered sugar remains very large compared with that of the combination, this latter will remain almost constant in amount and equal amounts of sugar will be hydrolysed in equal times: the time curve will, in fact, be approximately a straight line. When, however, the amount of sugar present is materially reduced, the combination will more nearly follow the ordinary law of mass action. They point out that this explanation is in accord with all the known facts.

A considerable body of evidence is put forward in this communication which, in the main, confirms the conclusion arrived at by Horace Brown and Glendinning; but it will be shown that it is necessary somewhat to extend their argument.

A novel conception was introduced by Croft Hill in 1898,† who studied the action of maltase on maltose advisedly from the point of view that the change might be reversible. He not only showed that the product of hydrolysis (glucose) exercised a marked retarding effect but also that a change was producible in the concentrated solutions of glucose by maltase; in his opinion, this retardation was due to reversion and he suggested that maltose was reproduced. Subsequently, in 1903,‡ while upholding the view that the change was a reversible one, he came to the conclusion that the main product, at all events, was an isomeride

* 'Chem. Soc. Trans.,' 1902, vol. 81, p. 388.
† 'Chem. Soc. Trans.,' vol. 73, p. 634.
‡ 'Chem. Soc. Trans.,' vol. 83, p. 578.

of maltose. E. Fischer and the author* had meanwhile shown that the enzymes lactase and emulsin both acted reversibly.

English workers appear to have overlooked the work done by Tamman in 1892,† who studied with great care the action of emulsin on the two glucosides salicin and amygdalin, as well as that of invertase on cane sugar. He came to the conclusion that the rate of change was retarded by the products of degradation but could detect no sign of reversion; he even went so far as to express the opinion that enzymes did not act reversibly. He insisted, however, that their action was incomplete.

In dealing with problems of enzyme action, the probability that the extract used contains several enzymes cannot be lost sight of, since it is known that in yeast extract, for example, at least three sucroclastic enzymes are present together with a proteoclast in amounts which vary in different yeasts. It is at least conceivable that, in those cases in which reversion has been observed, the hydrolysis is conditioned by one enzyme and the synthesis by another. It will be obvious, therefore, that the field for investigation is a very wide one and that our knowledge of enzymes is of a most incomplete and unsatisfactory character.

The present communication deals with the problem of the rate at which change proceeds during the earlier period, when the products of hydrolysis are present in relatively small proportions.

The results obtained not only justify the extension to enzymes generally of the view put forward by Horace Brown and Glendinning in explanation of the action of diastase and invertase, but also make it possible to explain cases in which the departure from the law of mass action is in a direction contrary to that considered by these authors; the influence exercised by the products of change, which was not taken into account by them, will also be considered. The action of acids is compared with that of enzymes in a separate communication.

Preparation of Enzyme.—The enzymes considered are lactase, emulsin and maltase; the first two of these both condition the hydrolysis of milk sugar but the last affects only maltose. Unfortunately no way has been devised hitherto of working with a known amount of enzyme; the nearest approach to a satisfactory method is to prepare a fresh extract for each series of experiments under conditions as nearly uniform as possible. Far too little care is sometimes given to this operation, the importance of working rapidly and at a suitable temperature being commonly neglected. The following is a description of the methods adopted in the experiments referred to in this communication.

Lactase.—Ten grammes of Kephir grains were very vigorously

* 'Ber.,' 1902, vol. 35, p. 3144.

† 'Zeit. Physiol. Chem.,' vol. 16, p. 271.

agitated with 200 c.c. water, using the modified ice-cream mixer described by Moody:* after 4—6 hours, when the grains were reduced to a fine pulp, 5 c.c. of toluene was added and the closed vessel set aside at about 20° over night. The milky liquid was then decanted and rapidly filtered, filtration being repeated, if necessary, until a clear liquid was obtained. The extract thus prepared was a clear yellow liquid: as changes took place in it on standing, it was used without delay. It is probable that a nearly saturated solution of the enzyme is obtained by operating in the manner described, as the residue is still very active; thus on extracting it for a further period of 24 hours with 200 c.c. water, an extract was obtained of about one-fourth the activity of the first.

The unfiltered milky extract is much more active than the filtered, probably because it contains some enzyme in suspension; it may also be mentioned that repeated filtration seems to diminish the activity even of the clear liquid. Although lactase prepared by the ordinary methods, involving precipitation and subsequent washing with alcohol, possesses some power of inducing the hydrolysis of milk sugar, it is only feebly active in comparison with an extract prepared as above described. The following figures, showing the parts per hundred of sugar hydrolysed during the intervals indicated in 50 c.c. of a 5 per cent. solution of milk sugar, may be quoted in illustration:—

	1 hr.	4 hr.	24 hr.
Solution containing 20 c.c. filtered extract	4·8	16	45
„ „ 20 c.c. unfiltered extract ...	15	38	80
„ „ 40 c.c. „ „ ...	29	57	86
„ „ Kephir residues.....	17	45	74

As the extract affects cane sugar, it may be supposed that it contains invertase as well as lactase; there is also reason to believe that one or more proteoclasts are present.

Maltase.—The extract was prepared by merely grinding up with water, in presence of toluene at about 21°, yeast which had been dried as rapidly as possible—by spreading it in a thin layer on biscuit ware and exposing it to the atmosphere; the clear filtrate was used. Experience seems to show that the activity of such an extract depends on the temperature at which it is made and that different yeasts require to be extracted at different temperatures.

Toluene has been used as an antiseptic throughout the experiments, as it was found not to influence the activity of the enzymes, whereas chloroform had a distinctly retarding effect.

Rough experiments made to ascertain the temperature at which lactase exercises its maximum activity show this to be about 35°:

* 'Chem. News,' 1902 vol. 86, p. 230.

although the extract is still very active at 15°, above 38° the activity begins to lessen. The experiments with lactase and emulsin were made at 35—37°, those with maltase at about 30°. In making the experiments, a mixture in the desired proportions of the solution of the sugar and of the enzyme, in a closed flask, was kept at a known temperature in an incubator; samples were withdrawn from time to time: these were diluted so that they contained 0·2 per cent. of the sugar and their cupric-reducing power was then determined by the modification of Pavy's method described by Croft Hill. A simple calculation gave the proportion in which monosaccharide and disaccharide were present. It may be claimed that the results are comparable; probably they are affected by an error amounting to at least from 1 to 2 per cent. when the amount of sugar hydrolysed does not exceed about 70 per cent.

The proportion or weight of disaccharide hydrolysed during successive intervals having been determined, the results were plotted graphically; the coefficient of velocity K was also calculated for each result, on the assumption that the rate of change was proportional to the amount of hydrolyte which undergoes change.

The actions of lactase and emulsin on milk sugar and of maltase on maltose have been studied in the manner described, using solutions containing 2, 5 and 10 grammes of the hydrated disaccharide in 100 c.c. Typical series of results selected from a large number of a similar character are quoted in the following tables. In these, t is time from the commencement of the experiment, x the percentage of substance hydrolysed and K the velocity coefficient calculated from the equation $K = \frac{1}{t} \log_{10} \frac{100}{100 - x}$.

Lactase.—On contrasting the results given in the two following tables it will at once be obvious that the character of the change is dependent on the proportion which the enzyme present bears to the sugar. When, as in the case of Experiment I, the proportion of enzyme is relatively large, K falls throughout the whole period of change; whereas, when the proportion of enzyme is considerably reduced, equal amounts of sugar are changed in successive equal intervals of time until about 10 per cent. has been hydrolysed, the value of K increasing at first but afterwards falling rapidly as in the previous case.

2 *grammes Milk Sugar per* 100 *c.c.*

Table I.

100 c.c. Enzyme Extract.

t.	*x.*	*K.*
1 hr.	22·1	0·1085
2 hrs.	31·2	0·0812
3 ,,	38·9	0·0713
4 ,,	45·8	0·0665
5 ,,	51·5	0·0629
6 ,,	56·6	0·0604
10 ,,	69·0	0·0509
17 ,,	84·2	0·0471
23 ,,	92·4	0·0461
29 ,,	95·3	0·0457
38 ,,	98·0	0·0447

Table II.

40 c.c. Enzyme Extract.

t.	*x.*	*K.*
$\frac{1}{3}$ hr.	3·2	0·0423
$\frac{2}{3}$,,	6·4	0·0430
1 ,,	9·6	0·0438
$1\frac{1}{2}$ hrs.	13·2	0·0410
2 ,,	16·4	0·0389
3 ,,	20·8	0·0338
5 ,,	25·2	0·0252
23 ,,	47·6	0·0122
100 ,,	89·6	0·0082

The same result is brought out in Tables III and IV, which apply to somewhat more concentrated solutions of milk sugar.

5 *grammes Milk Sugar per* 100 *c.c.*

Table III.

100 c.c. Enzyme Extract.

t.	*x.*	*K.*
1 hr.	13·7	0·0640
2 hrs.	22·1	0·0543
3 ,,	27·2	0·0460
5 ,,	30·0	0·0310
24 ,,	51·0	0·0129

Table IV.

20 c.c. Enzyme Extract.

t.	*x.*	*K.*
1 hr.	1·6	0·00700
2 hrs.	3·2	0·00705
4 ,,	5·4	0·00602
23 ,,	21·4	0·00455
29 ,,	25·0	0·00430
47 ,,	32·7	0·00370
71 ,,	45·0	0·00365
144 ,,	49·6	0·00207

That similar results are obtained on working with different materials at different times is shown in Tables V and VI, which refer to experiments made at times 4 months apart.

10 *grammes Milk Sugar per* 100 *c.c.*

Table V.

Table VI.

Strong Enzyme Extracts.

t.	*x.*	*K.*	*t.*	*x.*	*K.*
1 hr.	13·0	0·0605	1 hr.	12·1	0·0560
2 hrs.	22·1	0·0543	2 hrs.	18·0	0·0431
4 ,,	33·7	0·0446	3 ,,	22·0	0·0360
6 ,,	38·0	0·0346	4 ,,	25·1	0·0314
24 ,,	55·5	0·0146	23 ,,	46·7	0·0119
48 ,,	64·5	0·0094	28 ,,	53·0	0·0117
			47 ,,	61·0	0·0087

Emulsin.—Tables VII—X refer to experiments made with emulsin; the conclusions to be drawn from them are precisely similar to those deduced from the experiments with lactase. It is to be noted that emulsin acts much less rapidly than lactase.

2 *grammes Milk Sugar per* 100 *c.c.*

Table VII.

0·2 gramme Emulsin.

t.	*x.*	*K.*
½ hr.	3·2	0·0282
1 „	4·8	0·0214
2 hrs.	6·4	0·0143
3 „	7·6	0·0114
4½ „	9·0	0·0091
6 „	10·0	0·0091
23 „	19·7	0·0041
29 „	22·0	0·0037
48 „	29·0	0·0031
53 „	30·7	0·0030
144 „	62·2	0·0029
264 „	77·5	0·0024

Table VIII.

0·4 gramme Emulsin.

t.	*x.*	*K.*
1 hr.	4·9	0·0218
2 hrs.	7·5	0·0169
4½ „	9·4	0·0095
6 „	10·6	0·0081
23 „	30·5	0·0069
29 „	35·0	0·0064
48 „	47·8	0·0059
53 „	50·0	0·0057
144 „	84·0	0·0055

5 *grammes Milk Sugar per* 100 *c.c.*

Table IX.

0·4 gramme Emulsin.

t.	*x.*	*K.*
1 hr.	1·0	0·00440
2 hrs.	1·8	0·00395
4 „	3·2	0·00352
6 „	4·5	0·00333
22 „	15·0	0·00320
46 „	25·5	0·00277
70 „	35·6	0·00271
167 „	54·7	0·00206

Table X.

0·1 gramme Emulsin.

t.	*x.*	*K.*
1 hr.	3·1	0·0137
2 hrs.	4·6	0·0102
3 „	6·0	0·00896
4 „	7·0	0·00790
6 „	8·8	0·00667
22 „	21·5	0·00478
46 „	34·7	0·00404
70 „	46·0	0·00382

Maltase.—Tables XI and XII refer to experiments with maltase and maltose and show that here again the same conclusions hold. K decreases steadily: when the proportion of enzyme is small the change is at first linear. It is of interest as confirming these results that when K is calculated from the value given in Croft Hill's tables* a similar series of decreasing values is obtained.

* 'Chem. Soc. Trans.,' 1898, p. 634.

Table XI.

Maltose, 5 per cent.

t.	*x.*	*K.*
1 hr.	7·3	0·0329
2 hrs.	13·9	0·0325
4 ,,	24·4	0·0304
7¼ ,,	31·7	0·0229
23 ,,	35·2	0·0082

Table XII.

Maltose, 10 per cent.

t.	*x.*	*K.*
1 hr.	4·7	0·0209
3 hrs.	11·7	0·0180
5 ,,	17·8	0·0170
23 ,,	23·9	0·0052
28 ,,	25·0	0·0045
47 ,,	31·4	0·0035

Concentration of Hydrolyte.—Although the experiments recorded in Tables I—XII furnish evidence that the rate of change decreases as the concentration of the sugar solution is increased, while the actual weight of sugar hydrolysed increases; as the experiments were made at different times and with different materials, it was necessary to carry out a strictly comparable series in which the amount of sugar present was the only factor varied. Table XIII shows the results obtained in experiments with relatively concentrated solutions of milk sugar and lactase in which the amount of enzyme present was quite small. As the concentration of sugar was increased, the rate of change diminished so that the fraction of sugar hydrolysed in a given time was inversely proportional to the amount of sugar present—so that a constant weight of sugar was changed independently of the concentration—a result which is in agreement with Adrian Brown's observations with invertase.

Table XIII.—Amount of Sugar Hydrolysed.

Solutions containing—	24 hours.		46 hours.		144 hours.	
	Proportion.	Weight.	Proportion.	Weight.	Proportion.	Weight.
10 per cent.	14·2	1·42	22·2	2·22	33·4	3·34
20 ,, ...	7·0	1·40	10·9	2·18	16·9	3·38
30 ,, ...	4·8	1·44	7·7	2·21	11·0	3·30

Table XIV relates to a series of experiments made with emulsin and milk sugar in which, however, the proportion of emulsin taken was not particularly small. Although the rate of change decreased as the concentration increased, the experiments afforded no evidence that a constant weight of sugar was hydrolysed in a given time independently of the concentration. In a second series, however, in which a considerably smaller proportion of enzyme (see Table XV) was used,

it was found that an approximately constant weight of sugar was hydrolysed whatever the concentration of the sugar.

Table XIV.—Amount of Sugar Hydrolysed.

Solution containing—	22 hours.		46 hours.		70 hours.	
	Proportion.	Weight.	Proportion.	Weight.	Proportion.	Weight.
10 per cent.	22	2·2	29	2·9	39·6	3·96
20 „	13	2·6	20	4·0	25·1	5·0
30 „	10	3·0	15	4·5	19·0	4·7

Table XV.—Amount of Sugar Hydrolysed.

Solution containing—	23 hours.		48 hours.		92 hours.	
	Proportion.	Weight.	Proportion.	Weight.	Proportion.	Weight.
10 per cent.	19·7	1·97	29·8	2·98	35·7	3·57
20 „	10·6	2·12	15·3	3·06	23·3	4·66
30 „	7·0	2·10	10·2	3·06	16·3	4·89

The conclusions drawn from the experiments recorded in the three preceding tables apply only to the concentrated solutions in which the proportion of enzyme present was small; in very dilute solutions, on the other hand, quite another effect is produced by changing the concentration. Table XVI relates to experiments in which the proportion of enzyme present was large relatively to that of sugar. It will be seen that on increasing the amount of sugar present there was nearly a proportionate increase in the amount hydrolysed, though the proportion hydrolysed, as well as the value of K, remained constant, a result in agreement with the law of mass action. It should be mentioned, however, that the error affecting titration in these experiments is somewhat large, owing to the influence of the proteids on the determination of the end point.

Table XVI.

Milk sugar per 100 c.c.	Amount changed in 3 hours.	K.
1·0 gms.	0·185	0·0296
0·5 „	0·098	0·0298
0·2 „	0·0416	0·0337

Concentration of Enzyme.—Lastly, Table XVII shows the effect of varying the amount of enzyme present. Experiments in this direction are limited by the uncertain nature of the material, as well as by the fact that it is the concentration of enzyme relatively to that of sugar which must be studied. The solution contained 5 per cent. of sugar. Solutions containing varying amounts of enzyme were obtained by diluting portions of one and the same stronger solution. The weight hydrolysed in a given time by varying amounts of enzyme was approximately proportional to the amount of enzyme, provided that the amount was not too large and also that the comparison was made during the earlier stages of hydrolysis before the secondary products began to exert a marked influence.

Table XVII.—Proportions Hydrolysed in 100 c.c. of a 5 per cent. Solution.

Solution containing	1·5 hours.	20 hours.	25 hours.	45 hours.	68 hours.
1 c.c. lactase.....	0·15	2·2	2·6	3·9	4·8
2·5 c.c. lactase....	0·4	5·8	6·8	10·2	12·6
10 „ „	1·6	23·3	—	38·6	48·5
20 „ „	3·2	45·8	54·5	—	—

Very small quantities of the enzymes lactase and emulsin were found to be capable of hydrolysing only a small amount of sugar: their action then ceased. This result affords very definite evidence in favour of the view that the products of hydrolysis—in this case glucose and galactose—are capable of combining with enzymes and of removing them from the sphere of action.

Table XVIII.—Proportions Hydrolysed in 100 c.c. of a 5 per cent. Solution.

Solution containing	24 hours.	144 hours.
0·66 c.c. lactase....	2·3	2·3
1·0 „ „	3·2	3·5
2·0 „ „	6·3	7·4
5·0 „ „	15·4	34·0

Summary and Discussion of Results—Nature of Enzyme Action.

From the results recorded in the foregoing tables, it is clear that two periods may be distinguished in the course of change—an earlier period, during which the change is a linear function of the time; and a later period, during which change proceeds at another rate (Tables

II, IV, VII, XI). The duration of these periods is conditioned by the relation which the amount of enzyme present bears to the sugar, the linear period disappearing when the amount of enzyme present is relatively considerable (Tables I, III, VIII). It will be noted that in the cases studied in which the action proceeds comparatively slowly, *e.g.*, hydrolysis by lactase, emulsin or maltase—K falls rapidly in value; whereas when the action proceeds rapidly, *e.g.*, hydrolysis by invertase or diastase—K increases.

To understand the origin of these differences, it is desirable to consider the subject of mass action somewhat in detail, even at the risk of repetition.

Changes such as that which cane sugar undergoes in an aqueous solution under the influence of a catalyst, in which, on account of its large relative mass, the part played by the solvent may be disregarded —known as changes of the first order—are assumed to follow the logarithmic law of mass action: it is supposed that the one substance only undergoes change and that the same fraction of the residue is changed in successive equal intervals of time, *i.e.*, the factor K deduced rom the equation $K = \frac{1}{t} \log \frac{S}{S-x}$ is a constant.

But this is an ideal conception, as it involves the assumption that the amount of catalyst functioning is so small as to be negligible. In actual practice, not only is the amount of enzyme used not inconsiderable but it is known that the rate of change is affected both by alteration in the concentration of the hydrolyte and in the proportion which the hydrolyte bears to the catalyst; moreover, the products of change are known to exercise an influence and in concentrated solutions reversion may take place.

In view of the proof afforded by E. Fischer's researches that a close relationship exists in configuration between the hydrolyte and the enzyme which alone affects it, there can scarcely be any doubt that hydrolysis by enzymes, in the first instance, involves the combination of the enzyme with the hydrolyte, as Horace Brown and Glendinning have assumed to be the case.

On this assumption, the rate at which the change proceeds will depend on the extent to which the combination of enzyme with sugar takes place as well as on the degree of readiness with which the compound breaks down.

The proportion of the total sugar present in solution combined with the enzyme and undergoing change at any one moment may be regarded as the *active mass* of the sugar. The conception of an active mass has already been introduced by Arrhenius* but in another connection—to account for the very rapid rise in the value of K, when cane sugar is hydrolysed by acids, occasioned by a rise in temperature, the increase

* 'Zeit. Phys. Chem.,' 1889, vol. 4, p. 226.

being far greater than can be accounted for by the increased "ionisation" or by the greater mobility of the molecules at the higher temperature. Arrhenius supposed that only a part of the total hydrolyte present was really concerned in the hydrolysis: this he termed the "active part." It was arbitrarily assumed that this was a fixed proportion of the total mass at any temperature and that the proportion increased rapidly as temperature rose.

On the hypothesis that the enzyme combines with the sugar, the active mass of the sugar will be that portion s of the whole S which is in combination with an amount of enzyme e: it will be convenient to speak of the combination $s + e$ as the *active system.*

It is to be supposed that several influences are at work in a solution containing enzyme and sugar: on the one hand, enzyme and sugar molecules seek to combine; but on the other, the water molecules also tend to unite with the sugar molecules—so that there is, so to speak, competition between the enzyme and the water for the sugar molecules, which results in the establishment of an equilibrium depending to some, though probably a very limited extent, on the proportion relatively to enzyme in which the water and sugar molecules are present.* The possibility that the products of change also compete for the enzyme must, however, not be left out of consideration.

It is necessary to consider separately four sets of conditions, viz.:—

Case I, in which, whatever the amount of sugar present, the quantity of enzyme is relatively small.

Case II, in which there is a difference from Case I inasmuch as the quantity of enzyme is relatively considerable.

Case III, in which the amount of enzyme diminishes as the action proceeds.

Case IV, in which the amount of sugar present is varied.

Case I.—As hydrolysis proceeds, assuming that the enzyme itself is not affected by the work it does, since the magnitude of the active system depends on the amount of enzyme present, it is obvious that in the initial stages, if the total amount of sugar present S be large compared with s, the enzyme will be in presence of enough sugar molecules to establish the maximum possible number of effective combinations: or in other words the magnitude of the active system will remain constant and the change will be expressible, as Brown and Glendinning have pointed out, as a linear function of the time. As hydrolysis

* The enzymes, it is to be supposed, are colloids, *i.e.*, substances which have but little affinity for water: the stability of the combination $s + e$ will therefore be but to a slight extent dependent on the proportion in which water is present; whereas, probably, in the case of a combination of a crystalloid, such as an acid, with sugar, the proportion in which the components of the system are in equilibrium would vary to a considerable extent with the concentration. This is known to be the case. The effect of acids is dealt with in a separate note.

proceeds, the amount S of sugar present decreases until it is no longer negligible compared with that of the active part s and hence the enzyme will no longer effect the maximum possible number of combinations: the proportion of sugar s undergoing change will then be a function of the total mass and the formation of active systems will be governed by the law of mass action. The rate of change will be a logarithmic function of the time.

This explanation is fairly in accordance with the observed facts in the case of invertase and diastase, the only enzymes hitherto experimented with, which have always been used in very small quantities.

Case II.—If, on the other hand, the quantity of enzyme used be relatively large, the active mass will be a function of the total mass from the very beginning of the experiment, so that the linear part of the change will escape notice. O'Sullivan and Tompson seem to have used a relatively large proportion of enzyme, and therefore it is easy to understand why they found the action of invertase to follow the logarithmic law, whilst subsequent observers using relatively small quantities of enzyme have noted departures from this law.

Case III.—When the amount of the enzyme does not remain constant but for some reason decreases, the magnitude of the active system will not only be a function of the amount of sugar but also of that of the enzyme; it will therefore be represented by an equation of the second order, in which both of two interacting substances decrease—as, for example, is the case in the interaction of an alkali and methylic acetate. Such an expression corresponds to a curve falling off from a logarithmic curve and therefore giving a series of decreasing values for K when this is calculated for the simple logarithmic law. In such a case, the change in its early stages will still be a linear function of the time, as the diminution in the amount of enzyme will not at first materially influence the magnitude of the active system.

Stated shortly, the ordinary equation of mass action

$$\frac{dx}{dt} = \mathrm{K}(\mathrm{S} - x),$$

where S is the total sugar and x the amount changed in time t, is applicable only to the period during which a constant relatively large proportion of enzyme is present together with a continually decreasing amount of sugar but uninfluenced by the products of change.

During the final period, when the products of change exercise an influence by withdrawing enzyme from the sphere of action,

$$\frac{dx}{dt} = \mathrm{K}(\mathrm{S} - x)(\mathrm{E} - y),$$

where E is the total enzyme, y the amount withdrawn in combination with the products in time t.

During the period when the proportion of sugar present is very large, x becomes negligible compared with S, so that

$$\frac{dx}{dt} = KS = k,$$

where k is a constant.

The apparent duration of the linear period must be affected not only by x becoming no longer negligible compared with S but also by the extent to which the products of change make their influence felt.

It may here be pointed out that Henri's formula combines in a single expression the linear and logarithmic periods, but does not take into account the last period during which the products of the change exercise a retarding influence.

All these points are brought out not only in the results given in this paper but also in those tabulated by Brown and Glendinning. Although these observers have called attention to the existence of a linear period followed by a "logarithmic" period, they have failed to point out the meaning which may be attached to the decrease in the values of K observed during the latter stages of hydrolysis, when the products exercise a marked influence.

Their experiments afford an example of such an influence. Thus in Table IV,* referring to the hydrolysis of a 3 per cent. solution of starch by diastase at 21°, the value of K will be seen to increase until about half the starch was hydrolysed and then to decrease. If the value after 60 minutes be made the new starting point and the values be recalculated in the manner adopted by Brown and Glendinning,† the following figures are obtained :—

Old time units.	New time units.	K_0.
60 minutes	—	—
80 ,,	20 minutes	458
100 ,,	40 ,,	461
120 ,,	60 ,,	448
140 ,,	80 ,,	410

It is evident that whereas K_0 should be a constant if the period were "logarithmic," it begins to decrease after about 66 per cent. of the starch has been hydrolysed: showing that the influence of the products in removing enzyme has begun to make itself felt.

The action of invertase appears to be much less affected by invert

* *Loc. cit.*, p. 400.

† *Loc. cit.*, p. 393.

sugar and that of diastase by maltose than is that of lactase, emulsin or maltase by the products to which they respectively give rise. Consequently, for these latter enzymes, the linear period is of short duration (Tables II, IV, VII, XI) and the logarithmic period is barely perceptible, owing to the rapid reduction in the rate.

Case IV.—When the amount of enzyme and water is kept constant whilst that of sugar is increased, it may be supposed that the magnitude of the active system will increase until $s+e$ reaches a maximum, a definite equilibrium being established between enzyme, sugar, and water, the whole of the enzyme, perhaps, becoming combined with the sugar. It may be assumed that if the amount of sugar be further increased, the equilibrium will remain unaffected, notwithstanding that an addition of sugar is practically equivalent to a withdrawal of water.

But if $s+e$ remain unaltered, whatever the proportion of sugar present beyond a certain minimum, a constant amount of hydrolyte will undergo change in a given time, although the proportion changed as also the value of K will decrease as the concentration is increased. This conclusion is entirely in agreement with the facts elucidated, especially by Adrian Brown, and with my own observations.

Lastly, it should be pointed out that in discussing the action of enzymes, besides taking into account the conditions affecting the formation of an active system and the part which such a system plays in hydrolysis, it is necessary to consider the relative stability or tendency to break down under the influence of water of the combination of enzyme and sugar in connection with the very different rates at which different enzymes condition hydrolysis. Generally speaking, the observed differences in the rate at which hydrolysis is effected may be conditioned by—

(*a*) Specific differences in the enzymes, *e.g.*, lactase as compared with emulsin, which both hydrolyse milk sugar.

(*b*) Differences in the configuration of the hydrolyte, *e.g.*, β-methyl glucoside and β-methyl galactoside, which are both hydrolysed by emulsin.

(*c*) Differences in the stability of the hydrolytes, *e.g.*, cane sugar as compared with milk sugar.

But it is very difficult to institute just comparisons, for whereas, in the case of acids, the effect of only a single substance on the variety of sugars may be contrasted, in studying the hydrolysis of sugars under the influence of enzymes, it is necessary in most cases to use a different enzyme for each sugar, so that positive data are not easily obtained. Experiments made under comparable conditions with acids to test their action on different sugars show that these are hydrolysed at very different rates: thus, for instance, whilst cane sugar is hydrolysed nearly 1000 times as rapidly as maltose, this latter under-

goes change about 1·3 times as rapidly as milk sugar. It is scarcely possible to doubt that even greater differences exist both in the affinity of the enzymes for the sugar and in the degrees of readiness with which the enzyme sugar systems break down than are known to hold in the case of acid sugar systems. It is important to keep these considerations in view in discussing the rate at which different enzymes effect hydrolysis.

It will be apparent from what has been said that there is no reason to suppose that the action of enzymes follows any other than a normal course; the difficulties which have been met with in interpreting such changes may be ascribed to the incomplete consideration of the numerous factors involved.

[D 1810, 8010; Q 1230.]

"Studies on Enzyme Action. III.—The Influence of the Products of Change on the Rate of Change conditioned by Sucroclastic Enzymes." By EDWARD FRANKLAND ARMSTRONG, Ph.D., Salters' Company's Research Fellow, Chemical Department, City and Guilds of London Institute, Central Technical College. Communicated by Professor H. E ARMSTRONG, F.R.S. Received April 5,—Read April 28, 1904.

D 1810 Hexoses—power of sucroclastic enzymes to combine with.
D 8010 Enzymes—activity of correlated with configuration of hydrolyte.
D 8010 } Emulsin, lactase, maltase—varying influence of glucoses and
Q 1240 } glucosides on their activity.

In the previous paper, it has been shown that, in order to explain the action of sucroclastic enzymes, it is necessary to assume not only that the enzyme combines with the hydrolyte but that it is also more or less affected by—and presumably combines with—the product of change.

At present there is but little information available bearing on this latter contention. The experiments to be described have been made with the object of ascertaining by direct observation whether and to what extent the action of a given enzyme is affected by one or more of the products formed under its influence. They establish very clearly the existence of a close relationship between the configuration of the hexose and the enzyme in those cases in which a retarding influence is apparent: it is difficult to explain such a result except on the assumption that the enzyme and hexose combine together in some intimate manner.